NMR Determination of concentration-switchable inclusion complex of a β-cyclodextrin derivative carrying a benzene group linked to a C,C-glucopyranoside spacer

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Abstract-This article describes the structure of the inclusion complex that a β-cyclodextrin derivative **1**, which includes a benzene ring tethered to a cyclodextrin moiety via a C,C-glucopyranoside spacer, forms when its concentration is 9 mM. As we have recently reported, **1** forms an intramolecular inclusion complex at a concentration of 2 mM. By contrast, detailed NMR structural analysis revealed that at a 9 mM concentration, **1** formed a symmetrical pseudo-dimer based on intermolecular inclusion complexiation between two molecules of **1**. Thus, the inclusion structures of **1** varied depending on concentration, which indicates that the structure of this β-cyclodextrin derivative could be "concentration-switchable."

Keywords: concentration-switchable inclusion complex; β-cyclodextrin conjugated with a guest molecule; intermolecular inclusion; intramolecular inclusion

Introduction

Cyclodextrins (CyDs) are among the main host molecules in supramolecular chemistry and are known to form supramolecular inclusion complexes with various kinds of guest molecules, based on host–guest interactions [1-6]. New aspects of host–guest chemistry have been recently discovered using CyD derivatives bound covalently to a hydrophobic (guest) molecule, i.e., CyD–guest conjugates. Several studies have reported the diverse inclusion complexes formed by CyD–guest conjugates [7-15]. These complexes are fabricated from the incorporation of some parts of the guest molecule into the cavities of the CyDs via intramolecular or intermolecular inclusion interactions. Intramolecular inclusion interactions lead to the formation of self-inclusion complexes, whereas intermolecular inclusion interactions lead to the formation of noncovalently-bound pseudo-dimers or pseudo-oligomers. These CyD-based inclusion complexes often have unique structures and interesting properties. Therefore, to construct the inclusion complexes from the CyD–guest conjugates and to clarify their structures are important in CyD chemistry.

We recently reported the preparation and inclusion structure of the β-CyD derivative **1**, in which a benzene group is tethered to the CyD moiety via a C,C-glucopyranoside group, as shown in Figure 1 [16]. Compound **1** was designed to be a carrier of the anti-cancer agent doxorubicin (Dox). Our detailed analysis of NMR spectral data on solutions of **1** at a 2 mM concentration helped us determine its inclusion complex structure. The benzene group of **1** is located over the primary side of CyD, and the sugar moiety over the benzene ring lies parallel to the axis of the CyD's cavity, as depicted in Figure 1 (I). This capped structure of **1** was a kind of self-inclusion complex resulting from the intramolecular hydrophobic interaction between the benzene group and the CyD cavity. We then showed that the formation of this self-inclusion complex considerably influenced the formation of the intermolecular inclusion complex between **1** and Dox.

We also performed NMR measurements for **1** at a 9 mM concentration and found that the ^{1}H NMR spectrum of **1** at this concentration differed from that at a 2 mM concentration. This evidence strongly suggested that the structure that **1** forms at a concentration of 2 mM is different from the one it forms at a concentration of 9 mM, which indicated that the inclusion complex structure of **1** might vary depending on the concentration of the conjugate. In CyD chemistry, it is often observed that the intermolecular inclusion behaviors of CyDs with guest molecules are different according to the molar ratios and concentrations [17-20]. Concerning the CyD–guest conjugates, the conjugate concentration sometimes affects their behaviors and changes their inclusion complex structures [21-24]. Since the change of the inclusion complex structures can lead to the expression of novel functions, to provide the CyD–guest conjugates possessing "concentration-switchable inclusion structures" is one of the current topics in CyD-based supramolecular chemistry.

This paper describes the detailed NMR structural analysis of **1** at a 9 mM concentration. By comparing the structure of **1** at a 9 mM concentration with our formerly reported structure of **1** at a 2 mM, we reveal the structural difference between the two concentration-dependent inclusion complexes of **1**.

Experimental section

The preparation of **1** was described in our previous paper (*15*). The one-dimensional (1D) ^{1}H NMR and two-dimensional (2D) rotating-frame nuclear Overhauser effect spectroscopy (ROESY) spectral measurements were performed with a 600 MHz JEOL ECA-600 FT-NMR spectrometer at 298 K. The 1D spectrum was obtained with 16 scans. The conditions for collecting the 2D ROESY spectrum were as follows: 5120 scans; mixing time 250 ms; spectral width 9000 Hz; data size 1024 (F_2)/ 256 (F_1); relaxation delay 1.5 s. The MALDI-TOF-MS spectrum was recorded by a Voyager DE STR spectrometer (Method: LDE1005; Mode: Linear; Laser: 2500; Positive mode). MALDI targets were prepared using 2,5-dihydroxybenzoic acid (DHB) as the matrix and implementing the dried droplet method. Compound **1** was dissolved in H_2O at a concentration of 9 mM. Then, 1 µL of **1** at a 9 mM concentration was deposited on the MALDI target and allowed to dry in vacuum. The DHB solution was prepared by dissolving the compound in H_2O (0.1% TFA, 1 mg/mL). Finally, 1 µL of the DHB solution was deposited on the MALDI target of the spot of **1** and allowed to dry in vacuum.

Results and discussion

Figures 2 (i) and (ii) depict the ^{1}H NMR spectra of **1** at a concentration of 2 mM and 9 mM in D_2O, respectively. Some of the ^{1}H NMR spectral patterns of the solutions at the two concentrations were different from each other, and we proceeded to assign in detail the ^{1}H NMR chemical shifts of the 9 mM solution of **1**. This assignment was performed by one- and two-dimensional NMR spectroscopic techniques, including ^{1}H NMR, ^{1}H-^{1}H COSY, and TOCSY (data not shown). Table 1 shows the ^{1}H NMR chemical shift assignments of **1** at a 9 mM concentration along with those of the 2 mM concentration solution that we previously reported. The ^{1}H NMR assignments clarified the differences in chemical shift of the proton signals of **1** observed at the two concentrations. Some specific proton signals attributed to the benzene ring and to the spacer at C2′–C9′ at a 9 mM concentration were shifted, compared with their counterparts at a 2 mM concentration. Figure 2 (iii) depicts the ^{1}H NMR spectrum of **1** at a 9 mM concentration in CD_3OD. In this solvent, where no inclusion association is expected to occur, the *ortho*-, *meta*- and *para*-proton signals of the benzene moiety were observed as three separate peaks. The ^{1}H NMR spectral pattern of **1** in CD_3OD was quite different from that observed in D_2O. This evidence strongly suggests that the benzene ring moiety of **1** is involved in some inclusion association in D_2O when the concentration of **1** is 9 mM. We also found that the supramolecular structure that **1** forms at a concentration of 9 mM in D_2O is different from the one it forms at a concentration of 2 mM in the same solvent.

To reveal the inclustion complex structure of **1** at a 9 mM concentration in D_2O, we performed a ROESY experiment. Figure 3 reports the ROESY spectrum of **1** at a 9 mM concentration in D_2O at 25°C. Some observed long-range ROE interactions (a–h) are summarized in Table 2. The conformational structure of **1** inferred from the observed ROE signals (a) and (d–h) is reported in Figure 4. The ROE signal (a) results from the interaction between the proton of the benzene ring and the Ha9′, which shows that the plane of the benzene ring is likely to be nearly parallel to the C9′–Ha9′ bond axis and to be in a coplanar conformation. The ROE signal (g) results from the interaction between the H4′ and the Hb9′. ROE signals similar to (a) and (g) were also observed in the previously reported ROESY spectrum of **1** at a 2 mM concentration. Therefore, the conformational structures around the sugar ring–C9′–benzene ring of **1** are essentially the same for the two concentrations of the conjugate.

The ROE signals (d–f) and (h) were characterized at a 9 mM concentration of **1**. Signal (d) resulted from the ROE interaction between Ha2′ and H5′, and signals (e) and (f) resulted from the ROE interactions of the Hb2′ with H7′ and H5′, respectively. On the other hand, no corresponding ROE signals were observed at all in the ROE spectrum of **1** at a 2 mM concentration. This evidence indicates that the conformational structures of the spacer part around the C1′–C2′ containing the amide bond are remarkably different at the two concentrations. Furthermore, signal (h) results from the ROE interaction between Ha9′ and the H4 on the glucose moiety of CyD linked with a C,C-glucopyranoside group. This means that the section of the structure that transforms from the sugar ring to the benzene ring bound to CyD is positioned outside the CyD cavity and that the benzene ring is conformationally parallel to the secondary side rim of CyD, as shown in Figure 4 (II). This structure is different from that formed by a solution of **1** at a 2 mM concentration, which is reported in Figure 1 (I). The proposed conformational structure of **1** at a 9 mM concentration reported in Figure 4 might be one of the stable structures that occur when the host–guest intramolecular interaction between the benzene ring and the CyD cavity does not take place.

The observed ROE signals (b) and (c) result from the interactions between the proton of the benzene ring and the H3 proton of the glucose moiety of CyD linked with a C,C-glucopyranoside group. The ROE observations mean that the benzene ring is located near the secondary side of the CyD cavity. This conclusion is also supported by the features of the ^{1}H NMR spectrum. The ROE signals (b) and (c) and the above-mentioned ROE signals (a) and (d–h) indicate that **1** at 9 mM forms some kind of oligomer based on the intermolecular interaction between the benzene ring and the CyD cavity. To identify the oligomer, we measured the molecular weight of the oligomer using MALDI-TOF MS spectrometry. When the sample including **1** described in Experimental section was measured, the MALDI-TOF MS

spectrum showed a [2M + Na] ion peak at m/z 2877 in addition to a [M + Na] ion peak at m/z 1452. There was no observation of the ion peak showing a bigger molecular weight than the [2M + Na] ion peak. Therefore, we could confirm that a dimer from **1** was formed as the result of the intermolecular inclusion complexiation between two molecules of **1**. Furthermore, the ^{1}H NMR spectrum of **1**, see Figure 2 (ii), strongly indicates the formation of a single structure, so we concluded that a symmetric pseudo-dimer structure formed at a 9 mM concentration, whereby each of the two benzene ring units cap the secondary side of the CyD cavity of the other member of the **1**–**1** dimer, as shown in Figure 5 (III). At a 9 mM concentration, the intermolecular interaction increases and promotes the formation of the intermolecular inclusion complex from two molecules of **1**.

The molecular modeling of the pseudo-dimer of **1** is reported in Figure 6. Molecular modeling calculations were performed using the molecular force field method with MMFF94 which was installed on the Avogadro Application Ver. 1.1.1. Stabilization energy (ΔE) upon formation of the pseudo-dimer of **1** was calculated to be -274 kJ/mol, according to Eq. $\Delta E = E_{dimer} - 2 \times E_{monomer}$, where E_{dimer} and $E_{monomer}$ represent optimized total potential energies of the minimum pseudo-dimeric structure and monomeric capped structure from **1**.

Conclusions

We found that the β-CyD derivative **1**, which includes a benzene ring tethered to a CyD moiety via a C,C-glucopyranoside spacer, formed a symmetrically structured pseudo-dimer through the intermolecular inclusion complexiation between two molecules of **1** at a 9 mM concentration. The supramolecular structure of **1** at a 9 mM concentration was quite different from that observed at a 2 mM concentration. Thus, compound **1** is a molecule whose inclusion structure is concentration-switchable. We believe this study can provide the information as to the development of novel CyD–guest conjugates which have useful functions based on the concentration-switchable supramolecular structures.

References

1. Zheng, Y., Wyman, I.W.: Review: Supramolecular nanostructures based on cyclodextrin and poly(ethylene oxide): Syntheses, structural characterizations and applications for drug delivery. Polymers 8, 198/1–198/18 (2016)
2. Crini, G.: A history of cyclodextrins. Chem. Rev. 114, 10940–10975 (2014)
3. Guiou, S., Sollogoub, M.: Advances in cyclodextrin chemistry. In: Werz, D. B., Vidal, S. (eds.) In Modern Synthetic Methods in Carbohydrate Chemistry, pp 241–283. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (2014).
4. Lincoln, S.F., Pham, D.-T.: Cyclodextrins: from nature to nanotechnology. In Gale, P.A., Steed, J.W. (eds.) In Supramolecular Chemistry: From Molecules to Nanomaterials Vol.3, pp 954–981. John Wiley & Sons Ltd., Chichester (2012)
5. Wallimann, P., Marti, T., Fuerer, A., Diederich, F.: Steroids in molecular recognition. Chem. Rev. 97, 1567–1608 (1997)
6. Uekama, K., Hirayama, F., Arima, H.: Pharmaceutical applications of cyclodextrins and their derivatives. In Dodziuk, H. (ed) In Cyclodextrins and Their Complexes, pp 381-422. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (2008)
7. Benkovics G., Hodek O., Havlikova M., Bosakova Z., Coufal P., Malanga M., Fenyvesi E., Darcsi A., Beni S., Jindrich J.: Supramolecular structures based on regioisomers of cinnamyl-α-cyclodextrins–new media for capillary separation techniques. Beilstein J. Org. Chem. 12, 97–109 (2016)
8. Malanga, M., Darcsi, A., Balint, M., Benkovics, G., Sohajda, T., Beni, S.: New synthetic strategies for xanthene-dye-appended cyclodextrins. Beilstein J. Org. Chem. 12, 537–548 (2016)
9. Engeldinger, E., Armspach, D., Matt, D.: Capped cyclodextrins. Chem. Rev. 103, 4147–4173 (2003)
10. Harada, A., Hashidzume, A., Takashima, Y.: Cyclodextrin-based supramolecular polymers. Adv. Polym. Sci. 201, 1–43 (2006).
11. Liu, Y., Fan, Z., Zhang, H.-Y., Yang, Y.-W. Ding, F. Liu, S.-X., Wu, X., Wada, T., Inoue, Y.: Homodimerization and heteroassociation of 6-O-(2-sulfonato-6-naphthyl)-γ-cyclodextrin and 6-deoxy-(pyrene-1-carboxamido)-γ-cyclodextrin. *J. Org. Chem*. 68, 8345–8352 (2003).
12. Harada, A., Miyauchi, M., Hoshino, T.: Supramolecular polymers formed by cinnamoy cyclodextrins. J. Polym Sci. A Polym. Chem. 41, 3519–3523 (2003).
13. Tanabe, T., Usui, S., Nakamura, A., Ueno, A.: The stability of self-inclusion complexes of cyclodextrin derivatives bearing a p-dimethylaminobenze moiety. J. Incl. Phenom. Macrocycl. Chem. 36, 79–93 (2000).
14. Kuwabara, T., Shiba, K., Nakajima, H., Ozawa, M., Miyajima, N., Hosoda, M., Kuramoto, N., Suzuki, Y.: Host-guest complexation affected by pH and length of spacer for hydroxyazobenzene-modified cyclodextrins. J. Phys. Chem. A 110, 13521–13529 (2006).

15. Park, J.W., Lee, S.Y., Song, H.J., Park, K. K.: Self-inclusion behavior and circular dichroism of aliphatic chain-linked β-cyclodextrin-viologen compounds and their reduced forms depending on the side of modification. J. Org. Chem. 70, 9505–9513 (2005). Others references cited therein.
16. Yamanoi, T., Oda, Y., Katsuraya, K., Inazu, T., Hattori, K.: Synthesis, structure, and evaluation of a β-cyclodextrin-artificial carbohydrate conjugate for use as a doxorubicin-carrying molecule. Bioorg. Med. Chem. 24, 635–642 (2016).
17. Akita, T., Yoshikiyo, K., Yamamoto, T.: Formation of 1:1 and 2:1 host–guest inclusion complexes of α-cyclodextrin with cycloalkanols: A ^{1}H and ^{13}C NMR spectroscopic study. J. Mol. Struct. 1074, 43–50 (2014).
18. Akita, T., Matsui, Y., Yamamoto, T.: A ^{1}H NMR titration study on the binding constants for D- and L-tryptophan inclusion complexes with 6-O-α-D-glucosyl-β-cyclodextrin. Formation of 1:1 and 2:1 (host:guest) complexes, J. Mol. Struct. 1060, 138–141 (2014).
19. Nakamura, A., Inoue, Y.: Supramolecular catalysis of the enantiodifferentiating [4 + 4] photocyclodimerization of 2-anthracenecarboxylate by γ-cyclodextrin. J. Am. Chem. Soc. 125, 966–972 (2003).
20. Utsuki, T., Hirayama, F., Uekama, K.: Different photodimerization behaviour of tranilast in α-, β- and γ-cyclodextrin complexes: Cavity-size and stoichiometry dependence. J. Chem. Soc. Perkin Trans. 2 109–114 (1993).
21. Miyauchi, M., Hoshino, T., Yamaguchi, H., Kamitori, S., Harada, A.: A [2]rotaxane capped by a cyclodextrin and a guest: formation of supramolecular [2]rotaxane polymer. J. Am. Chem. Soc. 127, 2034–2035 (2005).
22. Miyauchi, M., Harada, A.: Construction of supramolecular polymers with alternating α-, β-cyclodextrin units using conformational change induced by competitive guests. J. Am. Chem. Soc. 126, 11418–11419 (2004).
23. Carofiglio, T., Fornasier, R., Gennari, G., Lucchini, V., Simonato, L., Tonellato, U.: Synthesis and spectroscopic properties of a water-soluble porphyrin-modified β-cyclodextrin compound. Tetrahedron Lett. 38, 7919–7922 (1997).
24. Self-association of a naphthalene-capped-β-cyclodextrin through cooperative strong hydrophobic interactions, González-Álvarez, M. J., Méndez-Ardoy, A., Benito, J. M., Fernández, J.M.G., Mendicuti, F.: J. Photochem. Photobiol. A 223, 25–36 (2011).

Table 1. Chemical shift assignments of ^{1}H NMR spectra of solutions of **1** at concentrations of 9 mM and 2 mM

Compound **1**	Proton	Chemical shift		Chemical shift change Upfield (+)/ppm Downfield (-)/ppm
		9 mM concentration	2 mM concentration	
CyD unit	H1	4.91 [A], 4.94 [G], 4.89, 4.90, 4.91, 4.92, 4.92	4.87 [d, 2.8], 4.89, 4.89, 4.90, 4.91, 4.91, 4.99 [d, 2.8]	
	H2	3.49 [A], 3.42 [G], 3.43, 3.45, 3.46, 3.47, 3.51	3.40, 3.41, 3.51, 3.52, 3.55, 3.57, 3.59	
	H3	3.73 [A], 3.58, 3.63, 3.69, 3.77, 3.79, 3.80	3.82, 3.82, 3.84, 3.88, 3.88, 3.90, 3.94	
	H4	3.23 [A: t, 9.6], 3.47 [B], 3.51, 3.53, 3.57, 3.74, 3.75	3.20 [A: t, 9.0], 3.46, 3.48, 3.49, 3.53, 3.54, 3.57	
	H5	3.55 [A-5], 3.69 [A-6], 3.71 [A-6], 3.56, 3.61, 3.63, 3.64, 3.65,	3.56, 3.61, 3.68, 3.68, 3.69, 3.69, 3.73	
	H6	3.67, 3.67, 3.68, 3.68, 3.73, 3.74, 3.75, 3.76, 3.77, 3.78, 3.79, 3.82, 3.83	3.63, 3.64, 3.67, 3.68, 3.69, 3.71, 3.75, 3.76, 3.77, 3.78, 3.785, 3.81, 3.82, 3.89	
C,C-Glucosyl unit	Ha2’	2.56 [d, 15.1]	2.54 [d, 15.8]	−0.02
	Hb2’	2.67 [d, 15.1]	2.60 [d, 15.8]	−0.07
	H4’	3.08 [d, 9.7]	3.08 [d, 9.6]	
	H5’	3.45	3.45	
	H6’	3.68	3.47	−0.21
	H7’	3.51	3.76	+0.25
	Ha8’	2.94 [m]	2.89 [m]	−0.05
	Hb8’	3.05 [m]	3.02 [m]	-0.03
	Ha		7.19 [bs]	
	Hb	7.12~7.20	7.21 [bs]	
	Hc		7.15 [bs]	
	Ha9’	2.95 [d, 14.4]	2.95 [d, 14.5]	
	Hb9’	2.75 [d, 14.4]	2.70 [d, 14.4]	−0.05

Table 2. ROESY interaction signals from a solution of **1** at a 9 mM concentration

ROESY signal	ROE Interactions
a	Benzene-H ↔ Ha9’
b	Benzene-H ↔ CyD-H3
c	Benzene-H ↔ CyD-H3
d	Ha2’ ↔ H5’
e	Hb2’ ↔ H7’
f	Hb2’ ↔ H5’
g	Hb9’ ↔ H4’
h	Ha9’ ↔ CyD[A]-H4

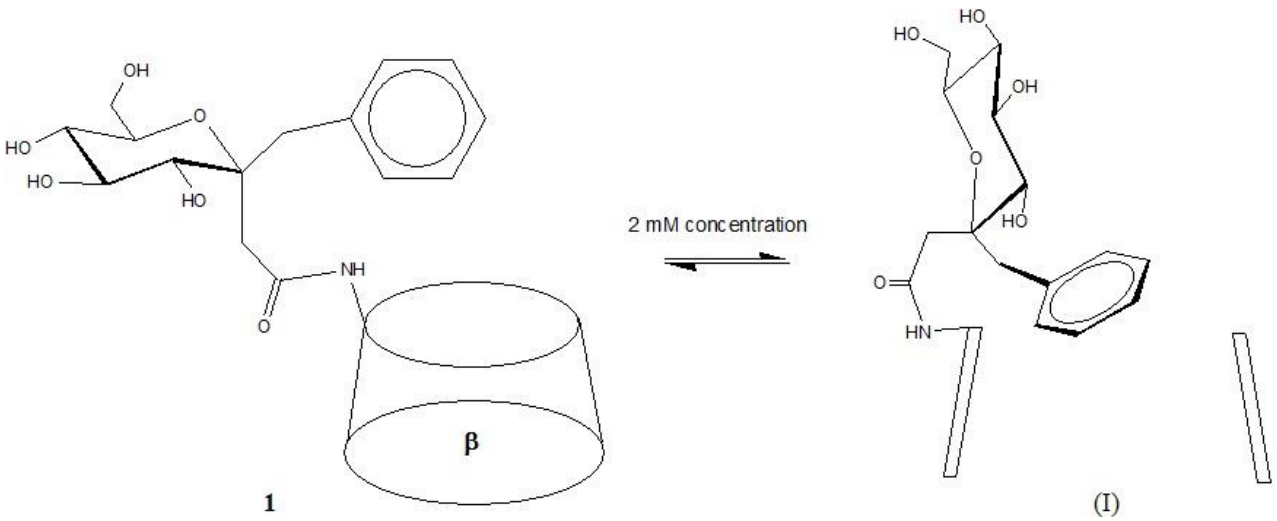

Fig 1. Self-inclusion structure of **1** at a 2 mM concentration

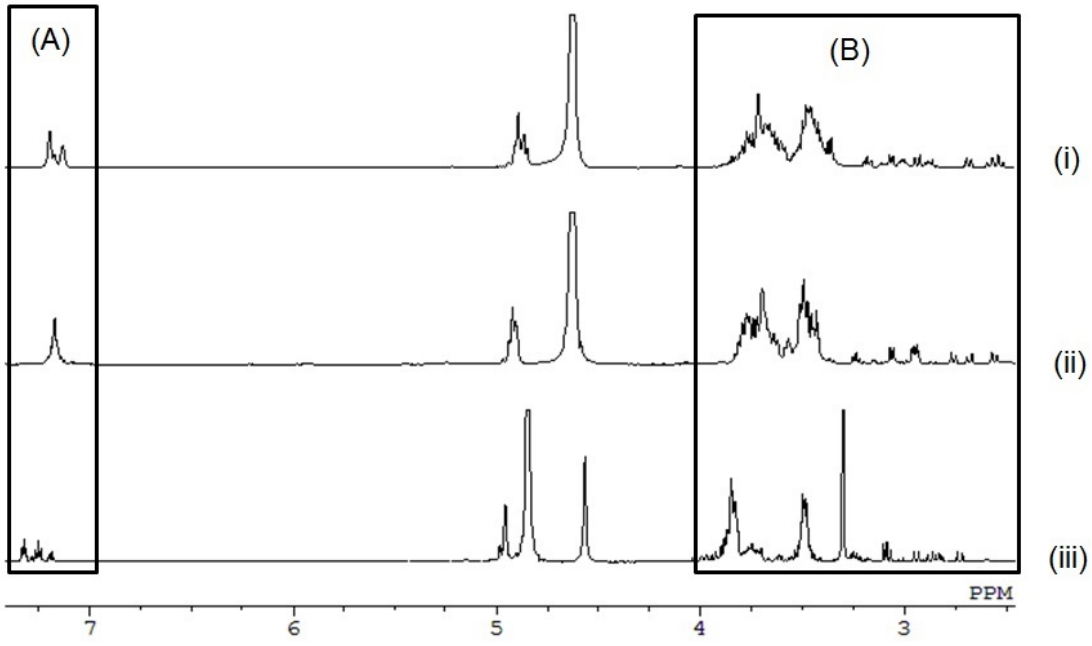

Fig 2 (a).

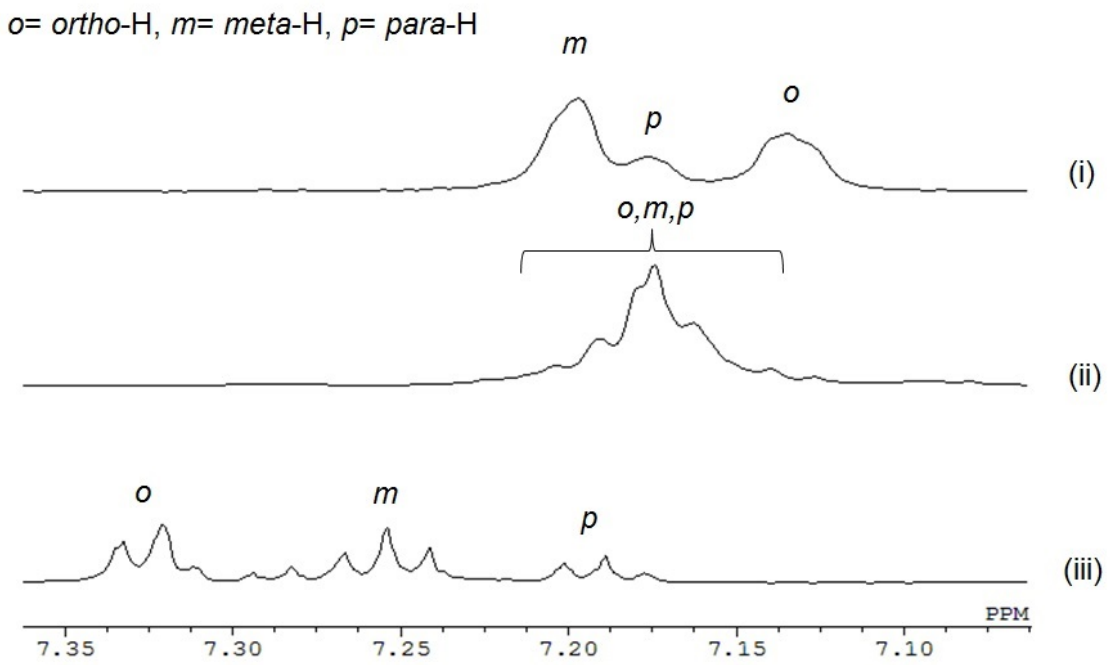

Fig 2 (b).

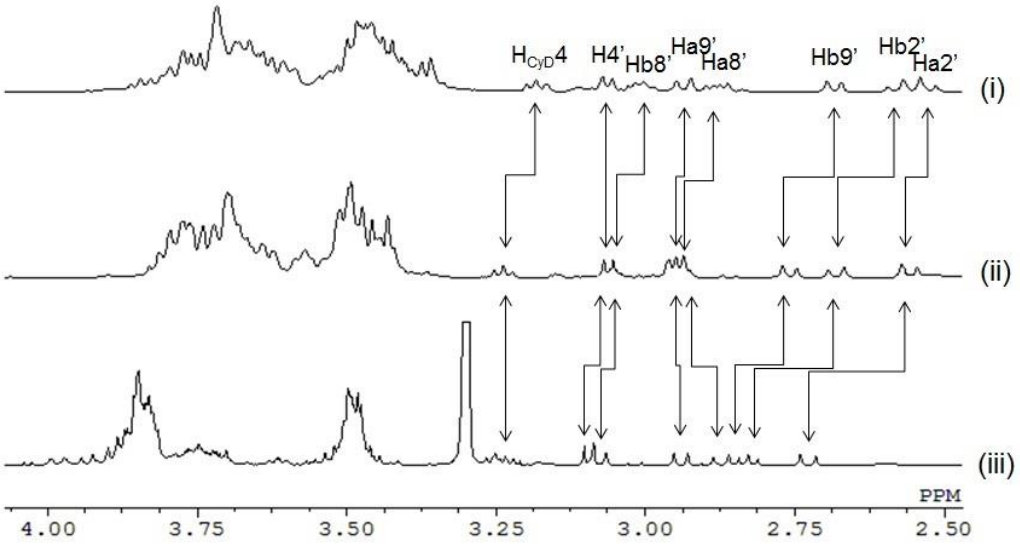

Fig 2 (c).

Fig 2. (a) ^{1}H NMR spectrum of **1**: (i) 2 mM concentration in D_2O; (ii) 9 mM concentration in D_2O; (iii) 9 mM concentration in CD_3OD. (b) Expansion of section (A) from (a). (c) Expansion of section (B) from (a)

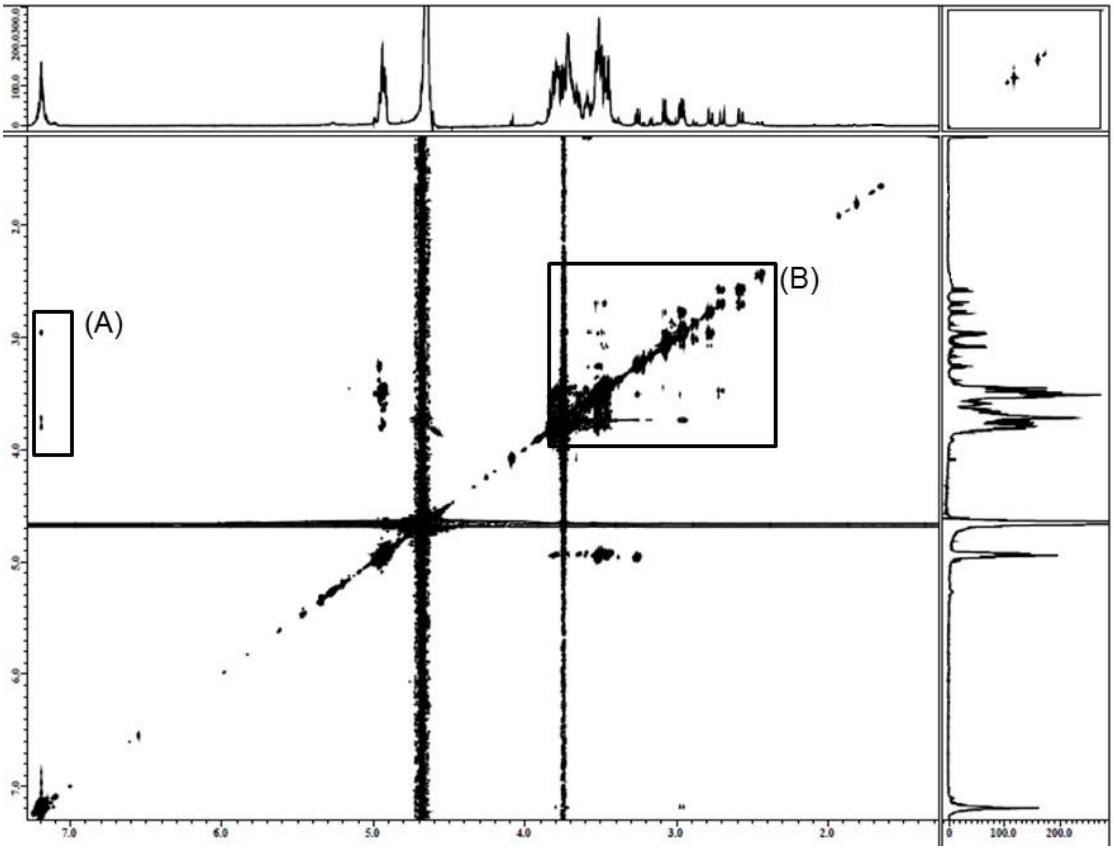

Fig 3 (a).

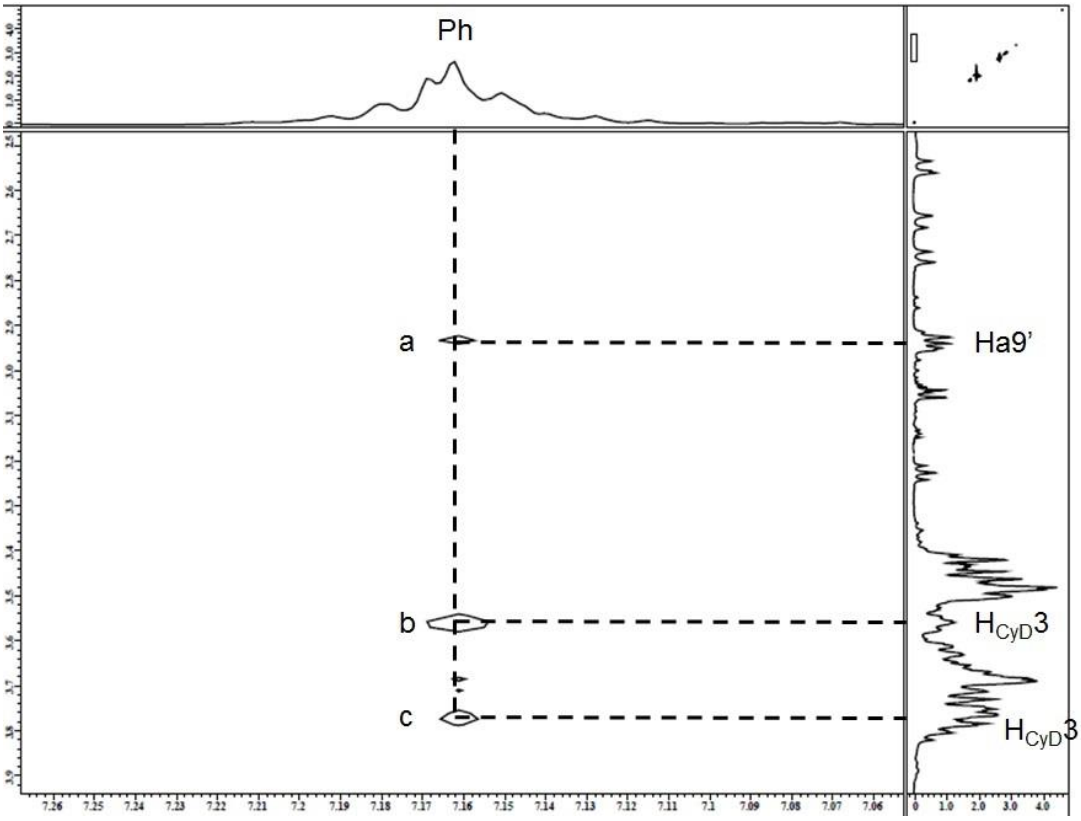

Fig 3 (b).

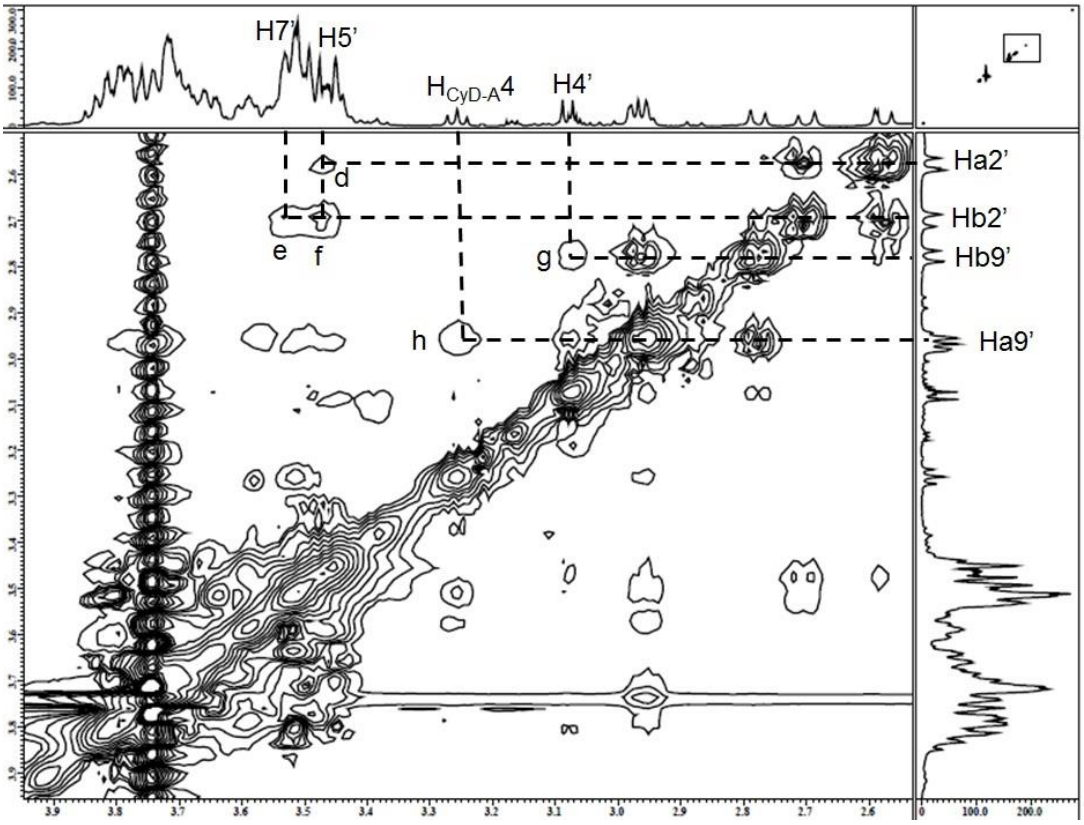

Fig 3 (c).

Fig 3. (a) ROESY spectrum of **1** at a 9 mM concentration: (b) Expansion of section (A) from (a). (c) Expansion of section (B) from (a)

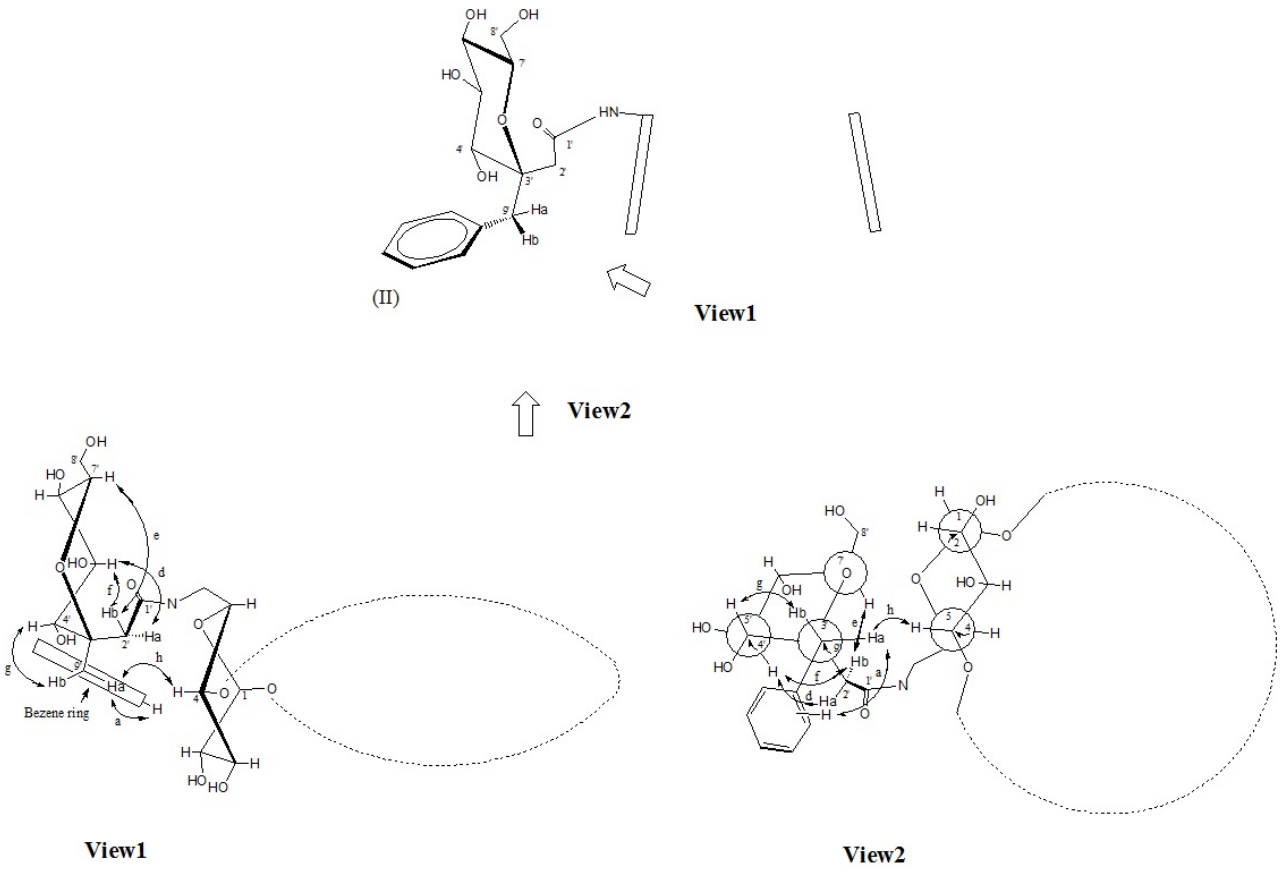

Fig 4. Monomeric conformational structure of **1** at a 9 mM concentration inferred from the ROESY interactions

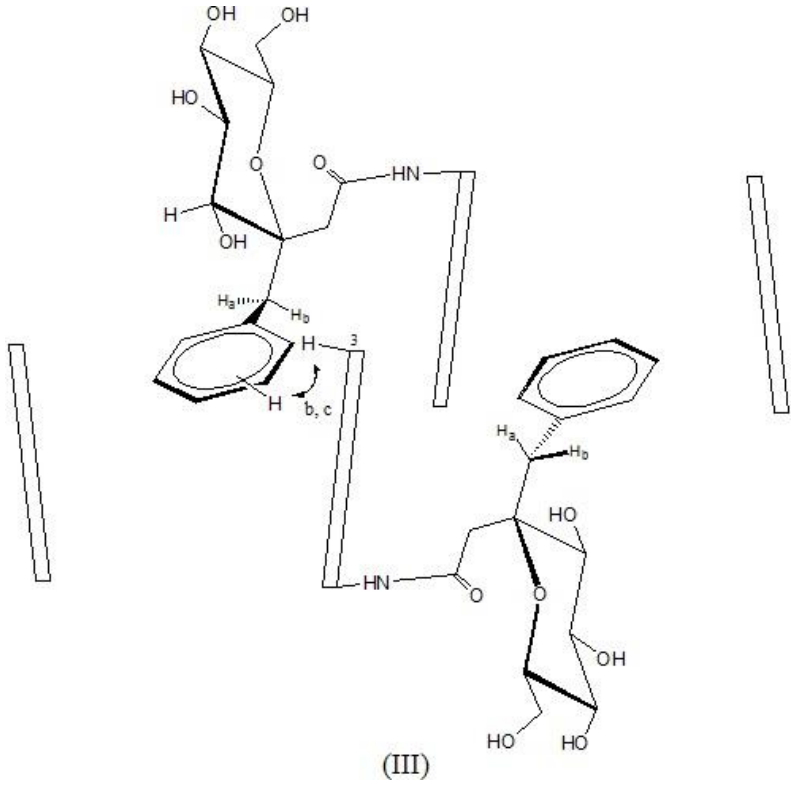

Fig 5. Speculated pseudo-dimeric structure resulting from the intermolecular inclusion complexiation between two molecules of **1**

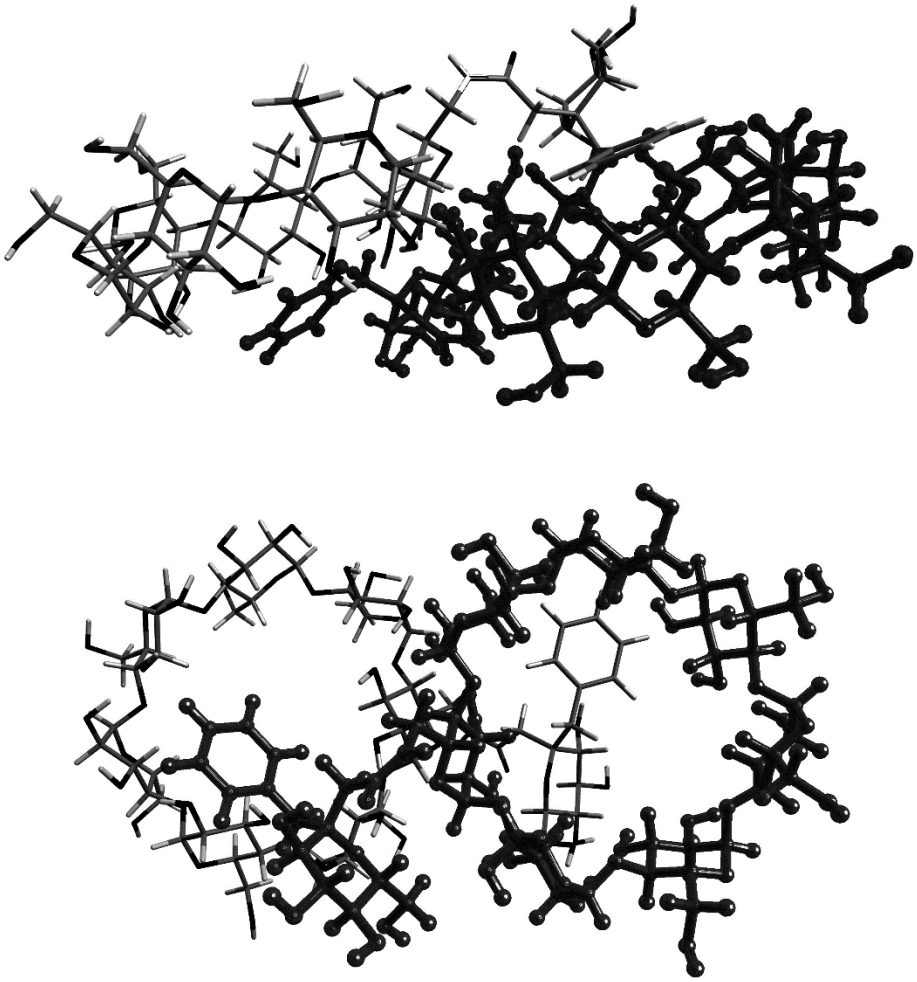

Fig 6. Molecular modeling of the pseudo-dimeric structure of **1**